Reverse phase HPLC of class I HLA eluted peptide ligands

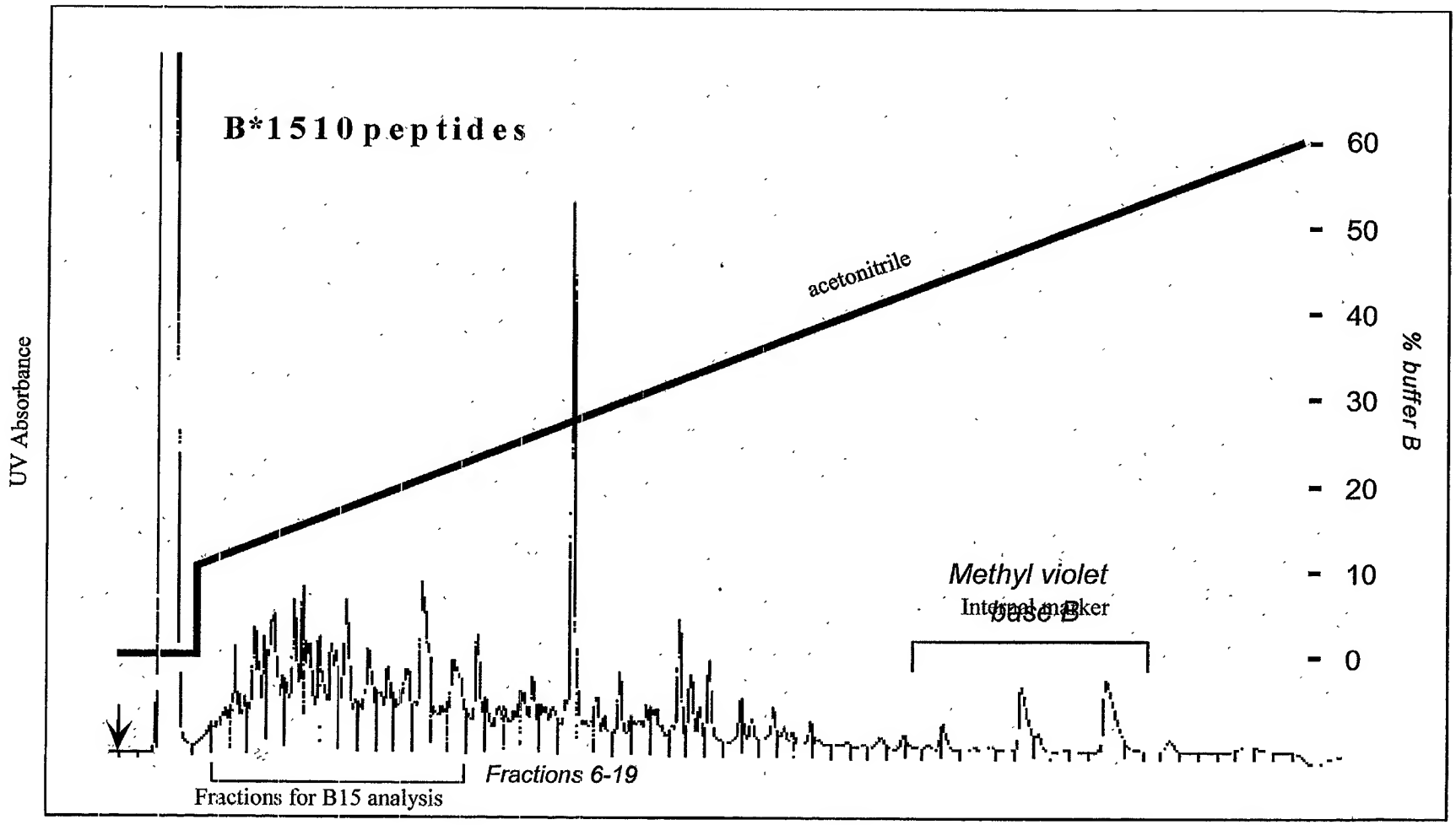


FIG. 1

Ion maps of peptides eluted from various B15 class I sHLA molecules. Mapping was accomplished with a nano-spray needle and an ESI mass spectrometer. The figure shows that the same ion peak is present in 3 of 4 B15 class I.

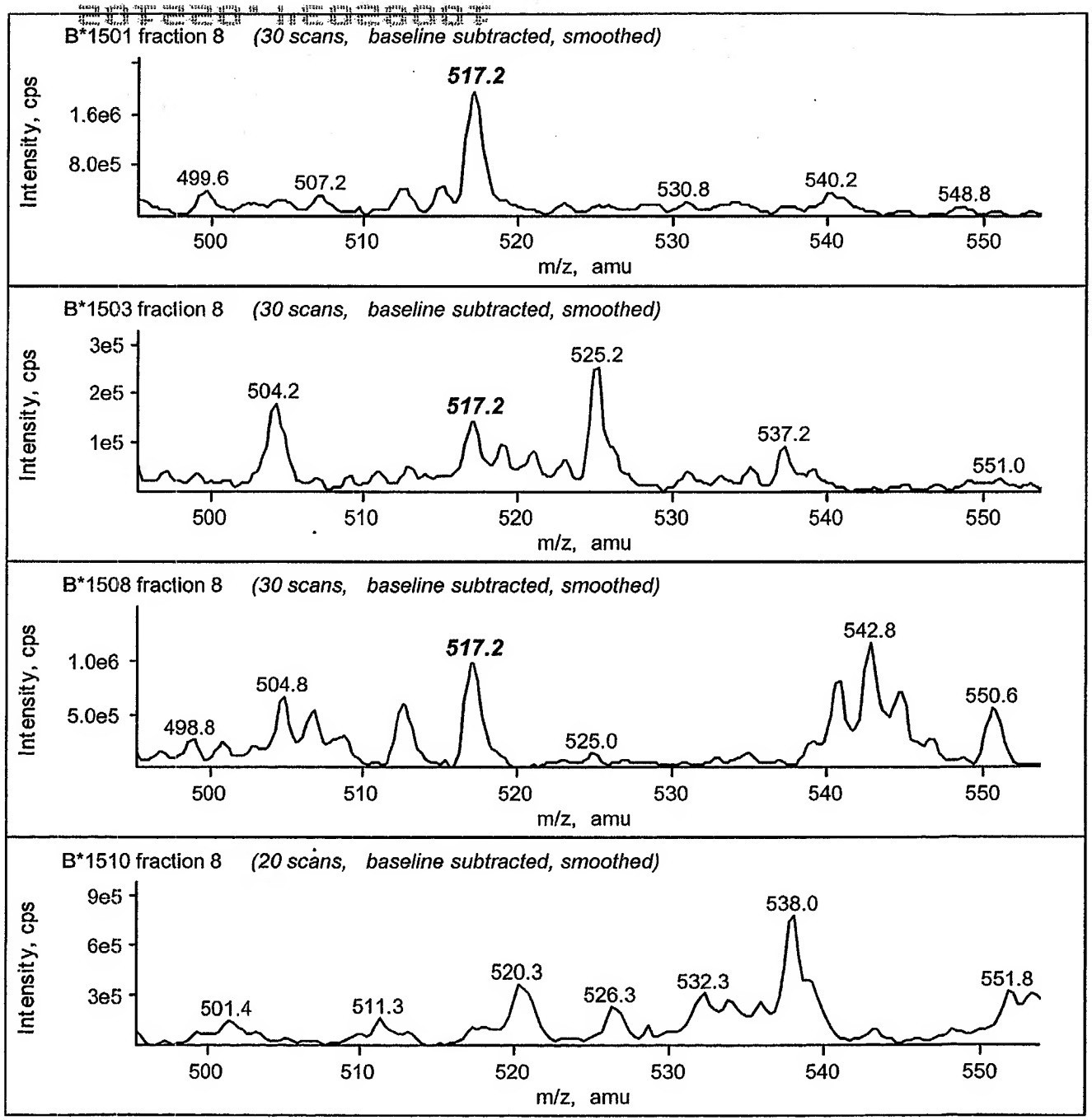


FIG. 2

MS/MS fragmentation-sequencing of ion 517.2 from the various B15 class I sHLA molecules. This data was accomplished by completing a second nanospray of the peptides in fraction 8 from the HPLC. This demonstrates how ions can be MS ion mapped and subsequently MS/MS sequenced. There is sufficient peptide present to do multiple MS/MS fragmentation runs. There is also sufficient peptide present to facilitate a submotif on fraction 8 or further separation in the event that two peptides had mapped at 517.2 in the ion map.

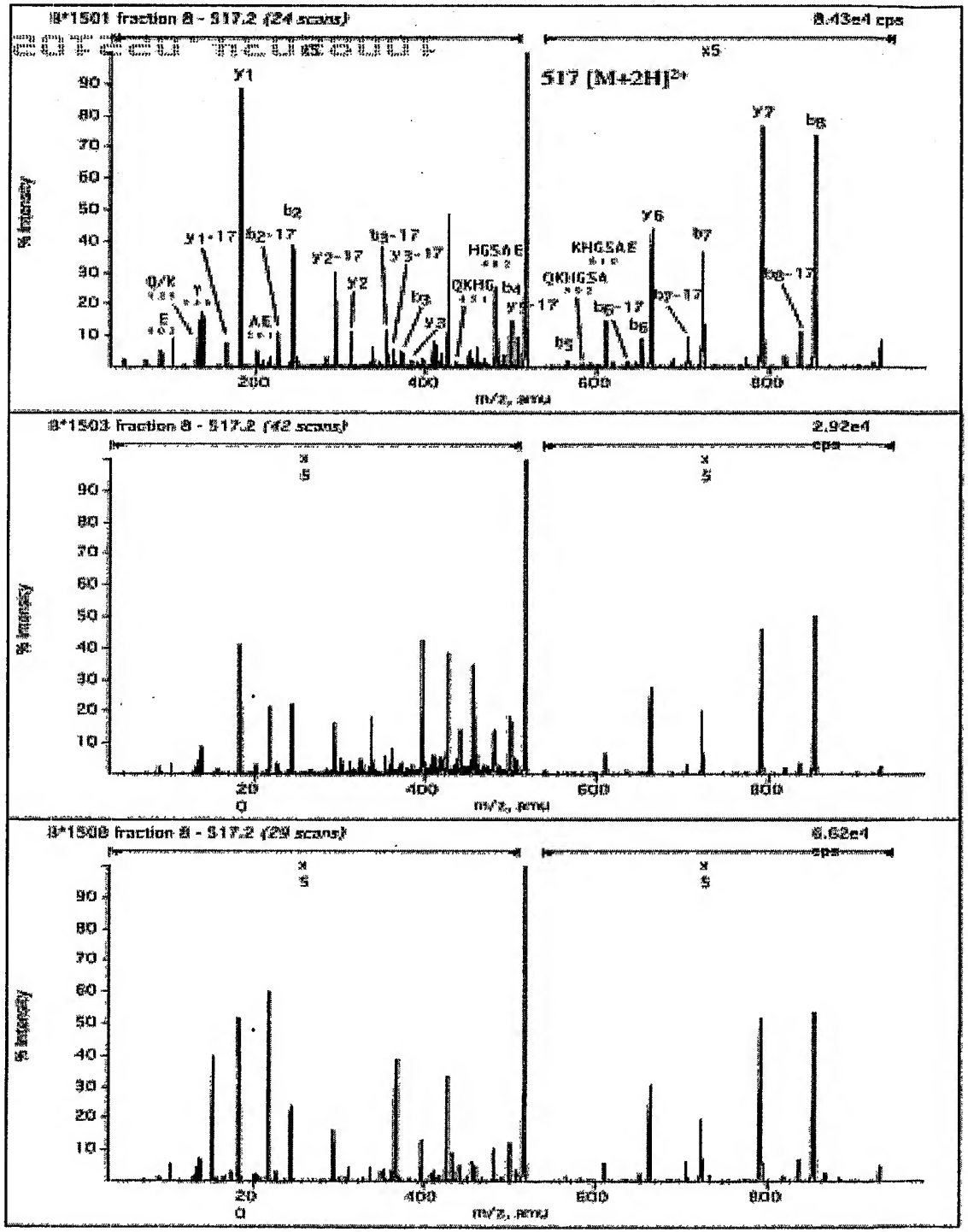
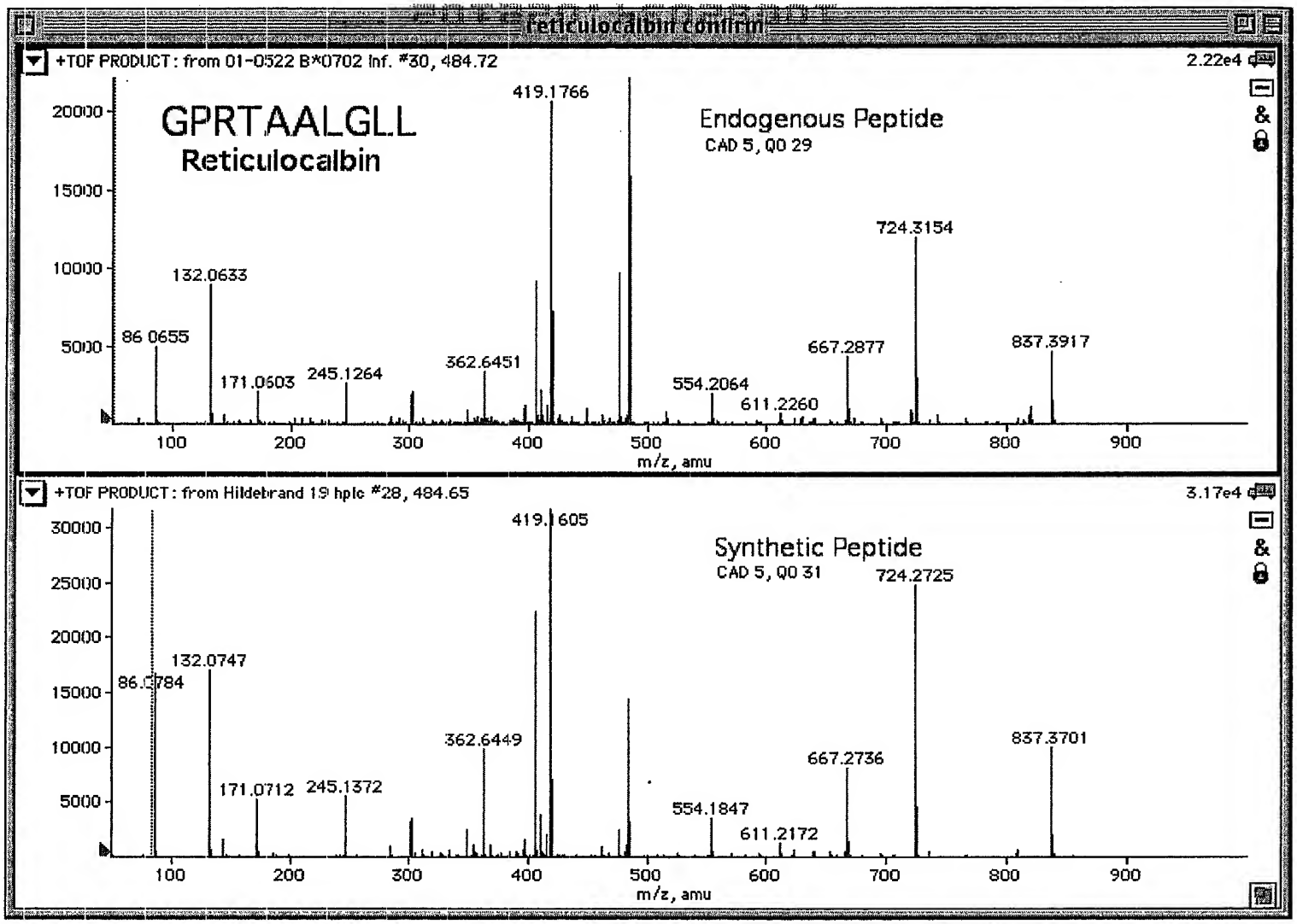


FIG. 3



sHLA B*0702 was secreted from HIV infected and uninfected cells. The ion maps of the peptides eluted from sHLA B*0702 in infected and uninfected cells were compared. Ion 484.72 was unique to the HIV infected cells. Ion 484.72 was subjected to MS/MS fragmentation-sequencing. We called GPRTAALGLL as the sequence of the ligand. We synthesized this peptide and found that it generated the same MS/MS fragmentation pattern as the ligand from HIV infected cells. This MS/MS data on a synthetic ligand matches our experimental data and validates the accuracy of our sequence.

FIG. 4

B*1508

	1	2	3	4	5	6	7	8	9
dominant	-	P	N	-	-	-	-	-	Y
	A	F							
		K							
		R							
		H							
		Y							
		I							
strong	-	-	-	E	H	R	-	-	F
			D						
			G						
			S						

--WDRHTXF
-----YT
XAZYEHTY
NGXAMHWTY
VPCGZZSY
TAZXHRGY
NQZHGSAEY
TG----AY
-P--GZDEV
NGYDGPNAGY
TPXGEPYZSY
FVSNHAY
SQFGGGSQY
SQFDHVTY
XAN--VT
VDX---Y
CPLSCFT
FLZAMZSTY
TVXDSZTHY
DPSGTZSGF
-----PGFY
-A-PHPMGY
AQTVGYGEY
------SVY
TGNCSGTGTY
AQVNPSXTY
TP--ARAPT
SPGAETRAXY
YX---RTF
YXG---GAF
-P---PSSGY
TP---GRMY
PMFDZZVY
AQEHGCAAZF
-M---GVHDY
YVS--RNZY

AQYAAGESFY
TPHTZHDEY
YM---FMY
MVGXXPAT
DPEYVSGHZF
VVACV---Y
PLA-N-HTY
VVAPITTGY
XAXYRRMY
PXAMQXYTY
-P-MPGXAY
--TZSNAY
MAAMVGVAVY
LPEQPLATY
FVTXNXEEY
GPZVMZHGY
FQARXTEY
AAAXV---VTY
XPEMGZFSY
YV--VR-VF
AAPVGAXESY
YVA---PAF
VGY--AHPGF
------SIY
SPIYTHAVAF
MPA--MVMAF
XA---SYIY
VGYVDDTQF
ZAINSVTSTY
YAIAGEMMAF
TARVXSVEY
MPAADYEVAF
AAFCG---XV
SPNEDXMZVF
VAATAGAVF
XLH--ET

ILGPPGSVY
XLGDVNMY
AP---XVSY
YMSGXYGTF
---AVVAZSY
MPAGYNNVY
XPVVPAAZTY
YMIDPSGVSY

B*1501

	1	2	3	4	5	6	7	8	9
dominant	-	Q	K	-	-	-	-	-	Y
		F							
		N							
		R							
		Y							
strong	-	M	P	P	G	-	-	-	F
	L	H	D	I					
	V	A	G						
			E						

--WDRHTXF
-----YT
ALGA--RGY
XS---VEY
AQFASGAGZ
-G--CDY
---ZARGY
ALNGRVTMY
DPHAPPZY
XAZVZMTAY
NQZHGSAEY
FGXACXATSY
APMARGZY
TG----AY
GQZZAVDF
TPXGEPYZSY
GQHASVXSY
FVSNHAY
---------SY
NPPAZZPN
-Q-DPPPDMZY
XQ--AGGZY
SQFGGGSQY
SQFDHVTY
--AAHVPPGY
FMDVGAPTVY
XAN--VT
AQM--SEY
CPLSCFT
FLZAMZSTY
TVXDSZTHY
AQAAPFAGY
----FGHY
ALW--PZF
VPHZNAY
-----GHGGY

FLZAMGSTY
GQYVVZPTY
PMFDPPZTF
XAVGHSGGTY
-----PTY
-----PSY
EPAMVXZCF
XAHTECPRGY
VQGPVGVZY
TGAPVSEEGY
VQXYYGSVV
GQPGAPXGGZY
GPPHNGXRAY
AAHWHVEAY
TPPTRRESY
FPTDRRSZF
YTGVSYXHF
AQASAPDAY
GQRKGAGSVF
VQYYXPF
ATGTAZNXNZY
VVACV---Y
PLA-N-HTY
XQYTVGYF
PLFGZTAGZY
A----ZXEY
VAGGW---F
SGAXDRAYZF
VQGPVGTDF
FQARXTEY
XAGFFXXEY
XQ----ZY
FQ----TX
TP---AZAF
VVATZNZZX

YMVT---F
APAV---VGY
------TGF
PVPNVRXNY
------TXSX
YMVCNAEEY
-----XRDXY
SLX-----F
ALGSZAXMPF
VGYVDDTQF
DVEGWMSZY
AQHPSAXRF
-----ZAY
GLGZTSAEF
NAXG--RESSF
TARVXSVEY
AAFCG---XV
XLH--ET
ILGPPGSVY
XLGDVNMY
VMGXTNANF
AVVTXZSDF
AMNPTNTVF
SQXAAGVDVF
-VFVSHTTF
XQGHHEMFY
PQGZMA--Y
HLTGNEATSF
YMIDPSGVSY

B*1510

	1	2	3	4	5	6	7	8	9
dominant	-	H	Y	P	-	-	-	-	L
		A							
		I							
		K							
		S							
		F							
		L							
		V							
		T							
strong	-	-	G	R	R	I	M	-	F
		D	D	V		L			
		E	K						
		M	E						
		N							

SVSXPHAP
APFTGGNGX
EHVASSPAL
HHAPCGVSX
NHAIVSTSV
GHZNSVTSV
SHZAPCTSV
FVARFVSX
HHSDGSVSL
MCZ-GMPAX
GHGANNDPAX
XHSZPAGPAX
XHVVS--VX
YHGSZNPEX
--------M
EHGXENGH
AHZAPPPTX
FTACZNPAX
SHAGAGXVX
GHXEGPXX
XHGGDHVX
YHHDXVX
MAGAWCRX
FH--XXX
EH---TVX
MAX----VV
------PVX
XHYDRNZX
------AXSV
XHWPVNEX
-H-----PVF
XHEVZPXHX
-HGCPGMPX
ETPEHAPVX
MXPGNSAXYX

FIG. 5

Pooled Peptide Motif

P1	P2	P3	P4	P5	P6	P7	P8	P9
	T	R	P					
	S	E						
		Q						
		M						
		Y						Y
		K						
		D						
		H						

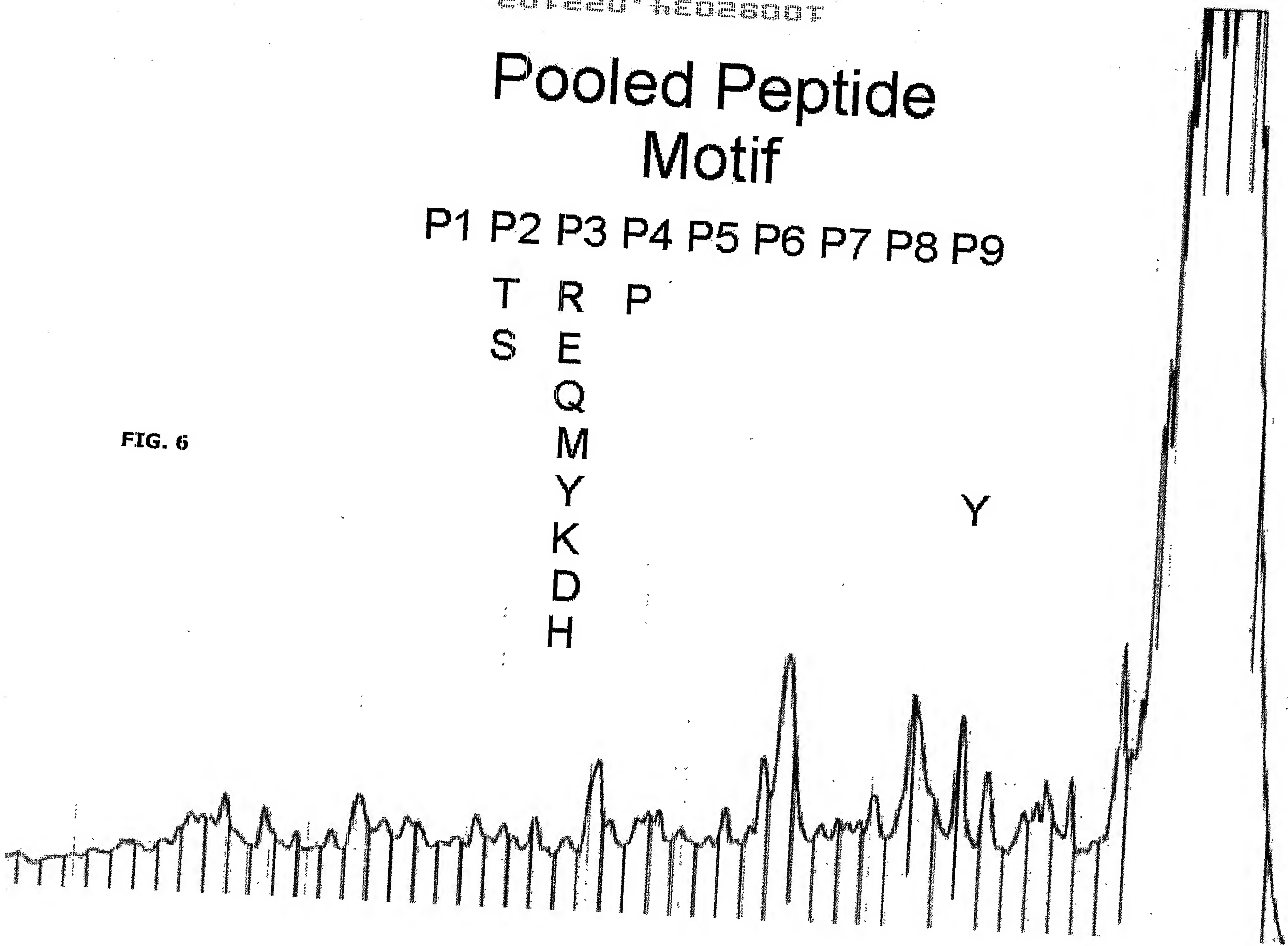


FIG. 6

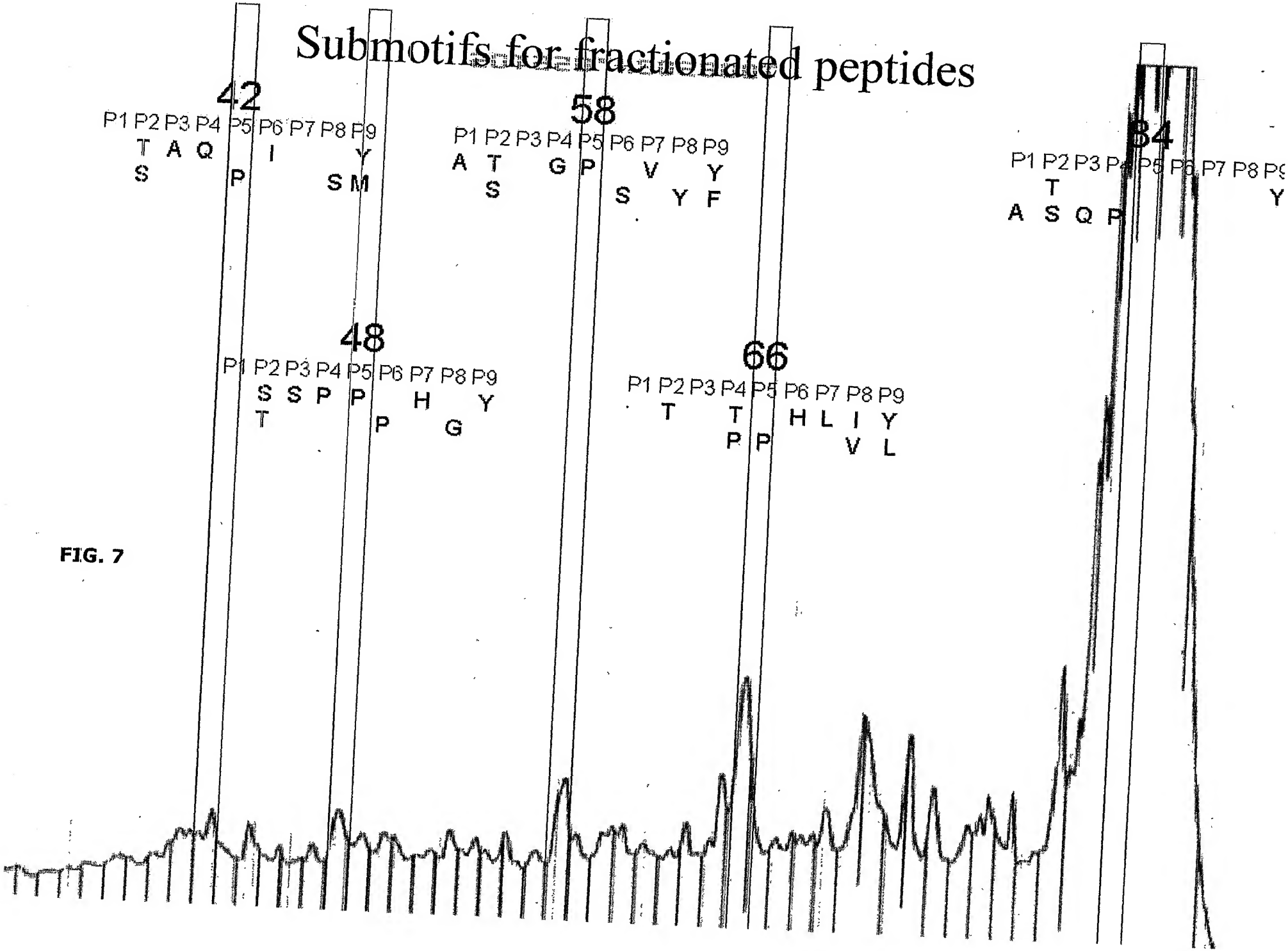

Submotifs for fractionated peptides
42
P1 P2 P3 P4 P5 P6 P7 P8 P9
T A Q I Y
S P S M
48
P1 P2 P3 P4 P5 P6 P7 P8 P9
S S P P H Y
T P G
58
P1 P2 P3 P4 P5 P6 P7 P8 P9
A T G P V Y
S S Y F
66
P1 P2 P3 P4 P5 P6 P7 P8 P9
T T H L I Y
P P V L
84
P1 P2 P3 P4 P5 P6 P7 P8 P9
T Y
A S Q P

FIG. 7

Narrowing search parameters using fraction motifs:

Ovarian Carcinoma Immunoreactive Antigen

MNGRADFREP	NAEVPRPIPH	IGPDYI**PTEE**	**ERRY**FAECND	**ESFWFRSY**PL
AATSMLITQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSYVKTCQE
KFKKLENSPL	GEALRSGQAR	**RSSPPGHYY**Q	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SA**PTGITDHI**	**Y**QGPDPNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNKYG
DTWDE				

Scanning with whole-pooled motif revealed 4 putative epitopes.

Ovarian Carcinoma Immunoreactive Antigen

MNGRADFREP	NAEVPRPIPH	IGPDYIPTEE	ERRYFAECND	ESFWFRSYPL
AATSMLITQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSYVKTCQE
KFKKLENSPL	GEALRSGQAR	**RSSPPGHYY**Q	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDPNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNKYG
DTWDE				

Scanning with fraction 48 peptide motif revealed 1 putative epitope.

FIG. 8

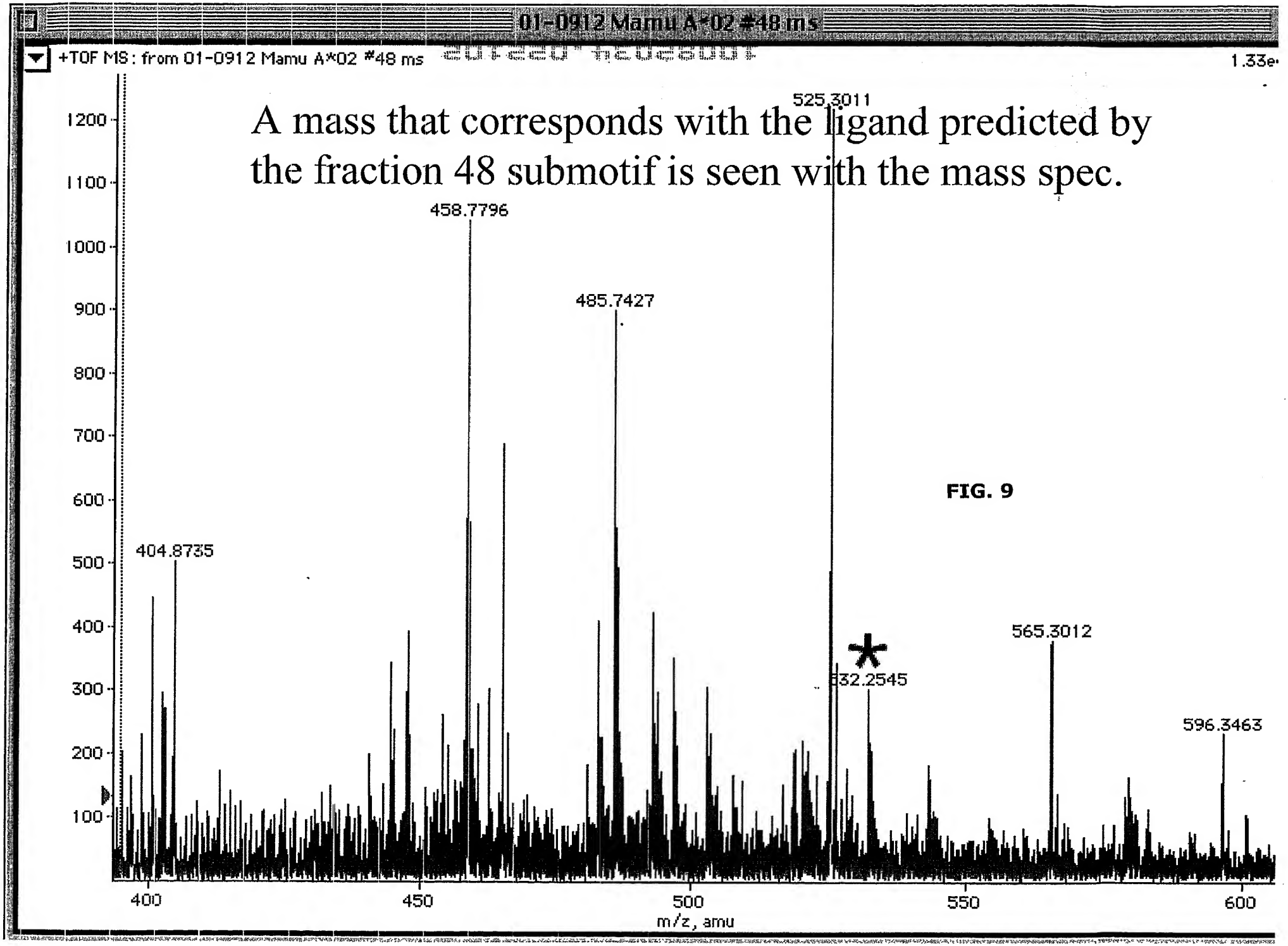
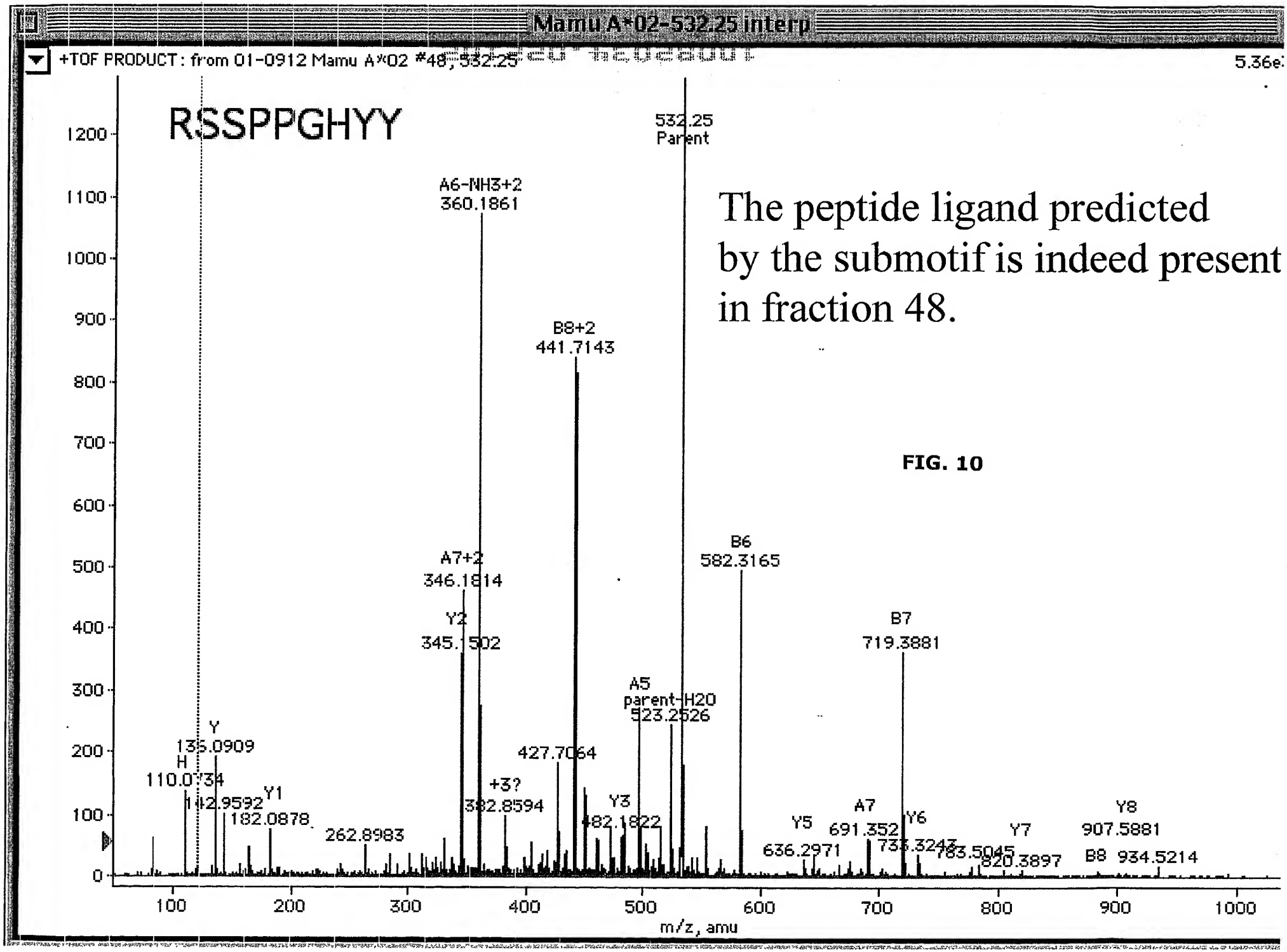

01-0912 Mamu A*02 #48 ms
+TOF MS: from 01-0912 Mamu A*02 #48 ms
1.33e
A mass that corresponds with the ligand predicted by the fraction 48 submotif is seen with the mass spec.
525.3011
458.7796
485.7427
404.8735
532.2545
565.3012
596.3463
1200
1100
1000
900
800
700
600
500
400
300
200
100
400
450
500
550
600
m/z, amu

FIG. 9



The peptide ligand predicted by the submotif is indeed present in fraction 48.

FIG. 10

Motif Data (Edman sequencing)

	1	%	fold	2	%	fold	3	%	fold	4	%	fold	5	%	fold	6	%	fold	7	%	fold	8	%	fold	9	%
Dominant 3.5 fold increase or more over prior round							F	9.20	11.18																	
							T	7.60	7.01																	
							N	6.20	4.11																	
							M	4.90	10.42																	
Strong 2.5-3.5 fold increase over prior round	K	3	50		R	53.80	2.57	Q	8.20	3.10	P	8.30	2.97												L	7.50
	R	15	50					K	5.40	2.67																
	S	10	40					L	5.10	3.47																
Weak 2.0-2.5 fold increase over prior round							A	5.80	2.08				M	4.90	2.28				T	7.70	2.12					
Trace 1.50-2.0 fold increase over prior round				Q	3.60	1.79	P	2.20	1.94	K	11.00	1.63	I	6.50	1.99							Q	11.40	1.93		
										S	6.00	1.68	F	4.20	1.83							N	5.70	1.55		
										V	5.30	1.99	H	1.80	1.67											

FIG. 11

FIG. 12

DESIGN OF HLA LIGAND/MOTIF DATABASE

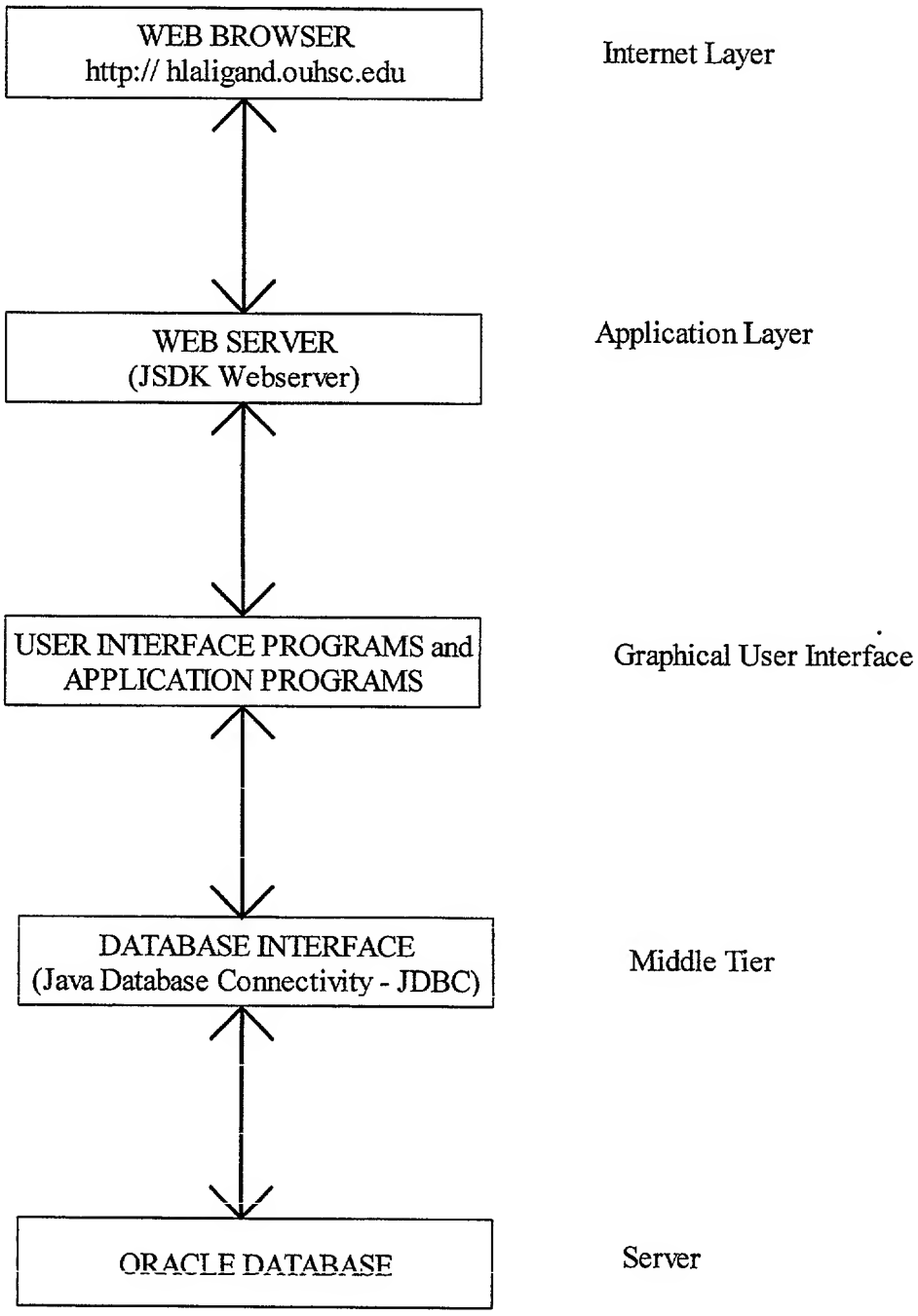


FIG. 13

Entity-Relationship (ER) Diagram for HLA Ligand/Motif Database

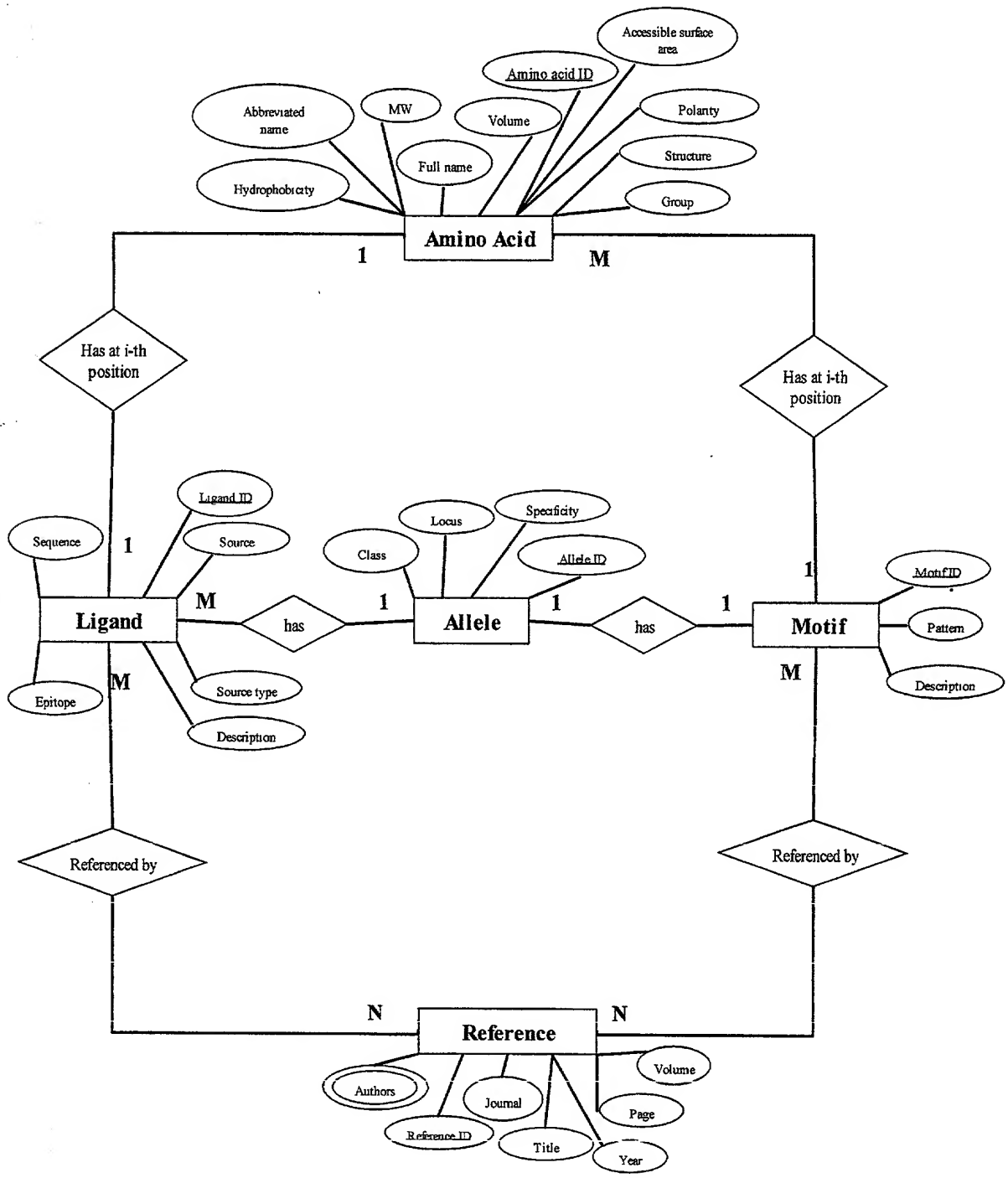


FIG. 14

UML Diagram for HLA Ligand/Motif Database